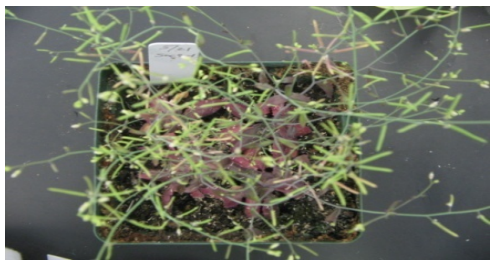
Identification of double and triple mutants of *sac9-1* and phospholipase D

Jane Pan and Mary Williams

- When stressed, plants induce molecular pathways, altering their morphology and behavior, and leading to stress tolerance. Phosphoinositides (PIs), regulators of these stress response pathways, maintain cellular events such as vesicle targeting, cytoskeleton function, and activate enzymes such as Phospholipase D (PLD). PLD produces PA, which has similar signaling roles as PtdIns(4, 5)P_2. Phosphatidylinositol(4, 5)P_2 (PtdIns (4, 5)P_2), a PI, accumulates in salt, cold, and osmotically stressed plants. The PtdIns(4, 5)P_2 signaling is terminated through the action of PtdIns(4, 5)P_2 phosphatases including SAC9. Unstressed *sac9-1* mutants accumulate elevated levels of PtdIns(4, 5)P_2 compared to wild type plants and are characterized by purple leaves, dwarfism, and closed stomata. We wanted to investigate whether these *sac9-1* phenotypes were caused directly by PtdIns(4, 5)P_2's effects on cellular pathways, or indirectly through its activation of the enzyme phospholipase D.



•Arabidopsis has 12 genes emcoding PLD. We examined the role of PLD in the *sac9-1* mutant by identifying a triple mutant of *sac9-1*, pldζ1 ,and pldζ2. It turns out that the phenotype of the triple mutant growing in soil is indistinguishable from that of *sac9-1*, indicating that the pool of PA produced by PLDζ1 and PLDζ2 is not a significant contributor to the mutant phenotype. We also produced a plant whose progeny will include a double mutant of PLDα1 and *sac9-1*, which will allow us to examine the role of PLDα1 in the *sac9-1* phenotype.